Th2 Cytokine mRNA Expression in Primary Cutaneous CD30-Positive Lymphoproliferative Disorders: Successful Treatment With Recombinant Interferon-γ

Hiroaki Yagi, Yoshiki Tokura, Fukumi Furukawa, and Masahiro Takigawa
Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan

Primary cutaneous CD30 (Ki-1)$^+$ large cell lymphoma (KiL) and lymphomatoid papulosis (LyP) type A are collectively termed as primary cutaneous CD30-positive lymphoproliferative disorders. We examined the cytokine profile of skin-infiltrating cells and the therapeutic efficacy of recombinant interferon-γ (rIFN-γ) in primary cutaneous KiL and LyP type A. By reverse transcriptase-polymerase chain reaction, mRNAs for interleukin-4 (IL-4) and IL-10 were detected in the dermis of skin lesions in all cases (three cases of KiL and four cases of LyP). In addition, tissue from one KiL patient transcribed IL-2 and IFN-γ messages, and one LyP patient showed IL-2 mRNA. In contrast, normal skin from ten healthy donors contained mRNA for IL-2 or IFN-γ, or both, but not for IL-4. Before the therapeutic trial of rIFN-γ, the response of skin lesions was assessed by a predictive skin test with local injection of rIFN-γ (0.5×10^6 Japan Reference Units [JRU; 1 JRU roughly corresponds to 4 NIH units]) for 3 consecutive days in two KiL and two LyP patients. Numbers of skin-infiltrating CD30$^+$ cells were decreased, and transcription of mRNA for IL-4 and IL-10 was downregulated after the skin test in one KiL and two LyP cases. One KiL patient showed no histologic response or change in mRNA expression. In the therapeutic trial, rIFN-γ (total doses of $1.2\text{-}4.0 \times 10^7$ JRU) was administered intravenously (n = 2) or locally (n = 2). In three patients who responded to the skin test, the lesions were objectively improved and the numbers of skin-infiltrating CD30$^+$ cells were markedly decreased after the therapeutic trial. No improvement was observed in one KiL patient who did not respond to the skin test. These findings suggest that the skin-infiltrating CD30$^+$ cells in KiL and LyP have a Th2 cytokine profile and raise the possibility that the administration of rIFN-γ improves the conditions by inhibiting cytokine mRNA transcription and proliferation of CD30$^+$ cells. *Key words: Ki-1$^+$ lymphoma/lymphomatoid papulosis/cutaneous T-cell lymphoma/anaplastic large cell lymphoma. J Invest Dermatol 107:827–832, 1996*

Primary cutaneous CD30-positive lymphoproliferative disorders (Willemze and Beljaards, 1993) form a spectrum of cutaneous lymphoma in which most or all of the anaplastic, large, sometimes Reed-Sternberg–like cells express CD30 (Ki-1 antigen) (Willemze *et al*, 1983; Kadin *et al*, 1985; Kadin, 1990; Davis *et al*, 1992; Willemze and Beljaards, 1993; Willemze, 1995). Despite the observation that some types of lymphomas in this group have a common activated T-cell origin (Kadin, 1985; Davis *et al*, 1992), the biologic behavior of atypical/tumor cells and thus the clinical course are quite variable. Lymphomatoid papulosis (LyP) represents a benign extreme (Macaulay, 1968), whereas high-grade CD30$^+$ large cell lymphomas (KiL) are aggressive (Willemze and Beljaards, 1993; Curco *et al*, 1994). LyP is a chronic, recurrent, self-healing papulonecrotic or papulonodular skin disease (Macaulay, 1968), and only type A is characterized by CD30 positivity (Willemze *et al*, 1983). Despite its malignant histologic appearance, less than 5% of cases of LyP precede or follow a malignant lymphoma, usually mycosis fungoides, Hodgkin's disease, or KiL (Beljaards and Willemze, 1992; Davis *et al*, 1992). Primary cutaneous and primary nodal KiL are distinct clinical entities that have identical morphologic features (de Bruin *et al*, 1993). Primary nodal KiL is common in childhood and adolescence and has a less favorable prognosis (de Bruin *et al*, 1993). In contrast to primary nodal KiL, primary cutaneous KiL generally occurs in adults, often shows partial or even complete spontaneous regression, and rarely develops extracutaneous lesions (Chan *et al*, 1989; Banerjee *et al*, 1991; Beljaards *et al*, 1993; Willemze and Beljaards, 1993). The t(2;5) chromosomal translocation, which is highly associated with primary nodal KiL, is not a common feature of primary cutaneous KiL (DeCoteau *et al*, 1996). Most of the reported cases of primary cutaneous KiL have had a favorable prognosis, with a 4-year survival rate of 90% (Beljaards *et al*, 1989; Chan *et al*, 1989; Kaudewitz *et al*, 1989; Banerjee *et al*, 1991; Beljaards *et al*, 1993; Willemze and Beljaards, 1993). Therefore, aggressive treatment is probably unnecessary for this type of cutaneous lymphoma (Beljaards *et al*, 1993).

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Reprint requests to: Dr. Yoshiki Tokura, Department of Dermatology, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431-31, Japan.

Abbreviations: JRU, Japan Reference Unit; KiL, CD30 (Ki-1)$^+$ large cell lymphoma; LyP, lymphomatoid papulosis.

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On the basis of distinct patterns of cytokine secretion, T-helper cell populations are categorized in both mice and humans into the Th1 cell subset, secreting interleukin-2 (IL-2) and interferon-γ (IFN-γ), and the Th2 cell subset, producing IL-4, IL-5, and IL-10 (Mosmann and Coffman, 1989; Romagnani, 1991; Paul and Seder, 1994). These two types of T cells and the cytokines derived from them counterregulate the biologic behavior of each other (Mosmann and Coffman, 1989). These subsets are the important components of T-cell responses in several diseases (Karpus *et al*, 1992; Clerici *et al*, 1993; Rapoport *et al*, 1993). The administration of Th1 cytokines such as IFN-γ is thus expected to improve disorders in which Th2 cells are pathogenic by counteracting Th2 cells and activating Th1 cells (Boguniewicz *et al*, 1990; Fushimi *et al*, 1996).

This article describes the Th2 nature of skin-infiltrating, atypical cells in primary cutaneous CD30-positive lymphoproliferative disorders. In three of four cases with primary cutaneous KiL or LyP type A, the local or intravenous injection of recombinant IFN-γ (rIFN-γ) improved the conditions and simultaneously downregulated Th2 cytokine mRNA transcription in skin-infiltrating cells.

MATERIALS AND METHODS

Patients Three patients with primary cutaneous KiL (cases 1 to 3; three males, mean age ± SD: 79.7 ± 4.9) and four with LyP type A (cases 4 to 7; three males and one female, age = 52.3 ± 28.2) were diagnosed on the basis of criteria reported previously (Macaulay, 1968; Willemze *et al*, 1983; Willemze and Beljaards, 1993). The skin lesions consisted of multiple tumors in cases 1 and 3, a solitary tumor in case 2, and self-healing multiple papules in cases 4 to 7. Extracutaneous dissemination of the disease or other types of lymphoproliferative disease were not demonstrated. All patients were seronegative for human T-cell lymphotrophic virus type I antibodies. The Southern blotting analysis of DNA from lesional skin (Ikuta *et al*, 1985) of two patients with KiL (cases 1 and 2) revealed rearranged nongermline bands with the use of a DNA probe for a constant region of T-cell receptor β chain (Cβ1). No clonal rearrangement was demonstrated in any LyP cases examined. No patients had received chemotherapy or radiation or local treatment of the skin lesions for at least 1 month before the study. Skin lesions of all KiL cases had been growing until this study and had not regressed spontaneously. Each lesion of LyP regressed spontaneously within a few weeks; i.e., it started as a small red papule that enlarged, then eroded, and finally healed, leaving a depigmented spot. Treatments and all examinations in this study were performed after informed consent was obtained.

Histologic and Immunohistochemical Studies Skin biopsy specimens were obtained from all patients. Half of the specimen was fixed in 10% formaldehyde solution and embedded in paraffin for light microscopy. The other half was fixed in periodate-lysine-paraformaldehyde solution, cryosectioned, and immunophenotyped with the three-step immunoperoxidase technique (Tokura *et al*, 1986). The monoclonal antibodies used in this study included anti-CD3, -CD4, -CD8, -CD20, -CD25, -CD45RA, and -human leukocyte antigen (HLA)-DR (Becton Dickinson, Mountain View, CA); anti-CD30 and -CD45RO (Dakopatts, Glostrup, Denmark); and anti-CD71 (Ortho Diagnostics Systems, Raritan, NJ). Lesions were considered positive for the marker if more than 50% of large atypical cells were stained.

Reverse Transcriptase Polymerase Chain Reaction (PCR) Analysis of Cytokine mRNA Expression Specimens were taken from tumors in KiL and papules in LyP. Skin samples from the trunk of ten healthy volunteers (four males and six females, mean age ± SD: 26.3 ± 9.2) served as controls. Dermal portions of the skin were resected from biopsy specimens under a microscope. Total RNA was extracted from the samples as described previously (Chomczynski and Sacchi, 1987). First-strand cDNA was reverse transcribed using each RNA sample and was amplified by PCR (Saiki *et al*, 1988) as described previously (Yagi *et al*, 1996). The primers used were as follows: IL-2, 5′ primer ATGTACAGGATGCAACTCCTGTCTT, 3′ primer GTCAGTGTTGAGATGATGCTTTGAC; IL-4, 5′ primer ATGGGTCTCACCTCCCAACTGCT, 3′ primer CGAACACTTTGAATATTTCTCTCTCAT; IL-5, 5′ primer GCTTCTGCATTTGAGTTTGCTAGCT, 3′ primer TGGCCGTCAATGTATTTCTTTATTAAG; IL-10, 5′ primer ATCAGCTGGACAACTTGTTG, 3′ primer GTCCTAGAGTCTATAGAGTC; IFN-γ, 5′ primer ATGAAATATACAAGTTATATCTTGGCTTT, 3′ primer GATGCTCTTCGACCTCGAAACAGCAT; and β-actin, 5′ primer TGACGGGGTCACCCACACTGTGCCCATCTA, 3′ primer CTAGAAGCATTGCGGTGGACGATGGAGGG.

The PCR products and DNA molecular-weight marker VI (Boehringer Mannheim GmbH, Mannheim, Germany) were separated in 2% agarose gels. The gel was stained with 1 μg ethidium bromide per ml, and amplified DNA bands were visualized with an ultraviolet transilluminator. The photographed bands of reverse transcriptase PCR products were scanned with a computer scanner (JX-330M; Sharp, Osaka, Japan). The density of the scanned band was quantified by image-analyzing software (NIH Image). The intensity of cytokine mRNA expression was estimated by calculating the relative density of cytokine PCR bands against β-actin in the same sample.

Evaluation of Therapeutic Effects of rIFN-γ The administration of rIFN-γ has been approved by the Ministry of Health and Welfare in Japan for the treatment of patients with cutaneous T-cell lymphoma (CTCL). Two × 10^6 Japan Reference Units (JRU; 1 JRU roughly corresponds to 4 NIH units) of rIFN-γ (Biogamma; Maruho Co., Osaka, Japan, and Suntory Ltd., Osaka, Japan; specific activity 5 × 10^6 JRU per mg of protein) was administrated intravenously in cases 1 (daily, 11 times) and 4 (daily, 20 times), and locally in cases 2 (daily, seven times) and 5 (twice a week, six times). The clinical efficacy of rIFN-γ administration was evaluated by comparing the severity of skin lesions before and after the treatment. The severity of KiL was expressed as the volume of each tumor. Eruptions of LyP wax and wane (Macaulay, 1968). The severity was thus assessed by enumerating eruptions and scoring the clinical appearance of each eruption (1 = mildly erythematous; 2 = moderately erythematous and papular; 3 = severely erythematous and papular) in three randomly selected areas (each approximately 100 cm^2) that initially contained at least five papules, and was expressed as the sum of the number of eruptions with the same clinical appearance multiplied by its score. Therefore, if one area contained three severely and five moderately erythematous and papular eruptions, the severity was (3 × 3) + (5 × 2) = 19. The assessment was performed every 7 d after rIFN-γ administration.

RESULTS

Histologic and Immunohistochemical Findings of the KiL and LyP Skin Lesions Biopsy specimens from the lesional skin revealed a dense lymphoid infiltrate in the dermis in all cases. The infiltrate in KiL was composed exclusively of large cells with abundant cytoplasm and a pleomorphic nucleus. In LyP, such large atypical cells were mixed with a moderate number of inflammatory cells, in particular neutrophils and eosinophils. Results of the immunohistochemical staining are summarized in **Table I**. In all cases examined, large atypical cells were positive for CD3, CD30, CD25, CD45RO, CD71, and HLA-DR. On the other hand, CD8, CD20, or CD45RA were not expressed in any case. Therefore, anaplastic large cells in these cases showed the morphology and expression pattern of surface antigens typically observed in KiL and LyP type A (Tokura *et al*, 1986; Beljaards *et al*, 1989, 1993; Kaudewitz *et al*, 1989; Parks *et al*, 1992).

Th2 Cytokine mRNA Expression by Dermal Infiltrating Cells Before exploring the cytokine profile of skin-infiltrating cells in KiL and LyP, we examined the background expression of cytokine mRNA in normal dermis **(Table II)**. Normal dermal samples from ten healthy donors contained mRNA for either IL-2 or IFN-γ, or both. On the other hand, mRNA for IL-4 or IL-5 was not detected in any sample. IL-10 mRNA was expressed in five samples regardless of the expression profile of other cytokines.

Figure 1 summarizes the mRNA expression for cytokines in the dermal portion of biopsy specimens in seven cases. All samples used in these experiments gave comparable amplified bands with the β-actin primers, indicating integrity of RNA. All biopsy specimens from the untreated lesions contained mRNA transcripts for both IL-4 and IL-10. IL-5 mRNA was detected in one case (case 3). Whereas five of seven cases (cases 1, 3, 5, 6, and 7) did not transcribe mRNA for IL-2 or IFN-γ, dermal portions contained mRNA for IL-2 in cases 2 and 4 and also IFN-γ in case 2, in addition to simultaneous transcription of mRNA for Th2 cytokines.

Decrease in $CD30^+$ Cell Number and Th2 Cytokine mRNA Transcripts by Local Injection of rIFN-γ Before the treatment, immunohistochemical and cytokine mRNA profiles were examined before and after the injection of rIFN-γ into lesions of the trunk in cases 1, 4, and 5 and in a solitary tumor of the neck in case 2, for 3 consecutive days (2.5 × 10^4 JRU/cm^2 of skin surface,

Table I. Immunophenotype of Large Atypical Cells[a]

Cases	Antigen									
	CD3	CD4	CD8	CD20	CD25	CD30	CD45RA	CD45RO	CD71	HLA-DR
KiL[b]										
1	+	+	−	−	+	+	−	+	+	+
2	+	−	−	−	+	+	−	+	+	+
3	+	+	−	−	+	+	−	+	+	+
LyP[c] (type A)										
4	+	+	−	−	+	+	−	+	+	+
5	+	+	−	−	+	+	−	+	+	+
6	+	−	−	−	+	+	ND[d]	ND	+	+
7	+	+	−	−	+	+	−	+	+	+

[a] Lesions were considered positive (+) if more than 50% of large atypical cells were stained.
[b] KiL, primary cutaneous CD30+ large cell lymphoma.
[c] LyP, lymphomatoid papulosis.
[d] ND, not done.

once a day). Whereas changes in clinical appearance of the eruptions were not overt in any case after a total dose of 1.5×10^6 JRU, the number of skin-infiltrating cells was reduced in response to rIFN-γ in cases 1, 4, and 5 **(Table III)**. Although both $CD3^+$ and $CD30^+$ cells were dramatically decreased in number, the effect of rIFN-γ was more apparent on CD30 than on CD3 expression, as assessed by the ratio of $CD30^+$ to $CD3^+$ cells (CD30/CD3). On the other hand, neither the number of skin-infiltrating cells nor the ratio of CD30/CD3 was changed in case 2. The density of each PCR band was quantified, and the intensity of mRNA expression in individual patients was expressed as the relative density of cytokine bands against the β-actin band for comparison between the samples. The densities of IL-4 PCR products were decreased, and the signals for IL-10 mRNA disappeared after the injection in cases 1, 4, and 5 **(Fig 1)**. This selective effect on cytokine transcription was remarkable in case 4, in which injections of rIFN-γ downregulated mRNA for Th2 cytokines and simultaneously upregulated mRNA for IL-2 and IFN-γ. The densities of bands for IL-4, IL-10, IL-2, and IFN-γ were at comparable levels before and after the injection in case 2 (data for post-treatment not shown), reflecting the immunohistochemical analysis. These findings indicate that a decrease in the CD30/CD3 value and disappearance of Th2 cytokine messages were critically related to the biologic activities of rIFN-γ. More important, as described in the next section, this skin test predicted the clinical efficacy of rIFN-γ.

Therapeutic Effects of rIFN-γ Recombinant IFN-γ was administered either intravenously or locally in the patients with KiL and LyP who had predictive skin tests. Three cases (cases 1, 4, and 5) responded well to rIFN-γ **(Fig 2)**; the severity scores after rIFN-γ administration in three lesions in each case were reduced to 42.0 ± 6.0%, 17.7 ± 4.7%, and 33.3 ± 11.1% (mean ± SD) of pre-treatment values, respectively. Reflecting the therapeutic effect of rIFN-γ, the number of skin-infiltrating cells and the CD30/CD3 ratio were reduced markedly after the treatment in cases 1, 4, and 5 **(Table III, Fig 3)**. After discontinuation of rIFN-γ, the severity score returned to the pretreatment level within several weeks. In case 2, on the contrary, the volume of tumor was not decreased and histopathologic findings were not changed by intralesional injections. Liver and renal function and blood electrolyte levels had been normal in all patients during the treatment and 6-month follow-up.

Table II. IL-2 and IFN-γ, But Neither IL-4 Nor IL-5 Cytokine mRNAs, Are Detected in Normal Dermis[a]

Individual	IL-2	IL-4	IL-5	IL-10	IFN-γ
1	−	−	−	+	+
2	+	−	−	−	+
3	+	−	−	+	+
4	+	−	−	−	+
5	+	−	−	+	+
6	−	−	−	+	+
7	+	−	−	−	−
8	+	−	−	−	+
9	+	−	−	+	−
10	+	−	−	−	+
Number of positive samples	8	0	0	5	8

[a] RNA was extracted from the dermal portion of skin biopsy specimens from ten healthy donors and analyzed by reverse transcriptase-PCR. +, detectable level by reverse transcriptase-PCR; −, below the detectable level.

DISCUSSION

This study indicates that Th2 cytokines are produced by $CD30^+$ anaplastic large cells. Two findings validate this notion. First, dermis infiltrated by $CD4^+CD30^+$ atypical cells always expressed mRNA for Th2 cytokines. This was not necessarily the case in normal samples. Second, the local injection of rIFN-γ, which counteracts Th2 cells preferentially, reduced the number of $CD30^+$ cells and simultaneously inhibited mRNA expression for Th2 cytokines. A literature search reveals no studies concerning the cytokine profile of skin-infiltrating atypical cells in primary cuta-

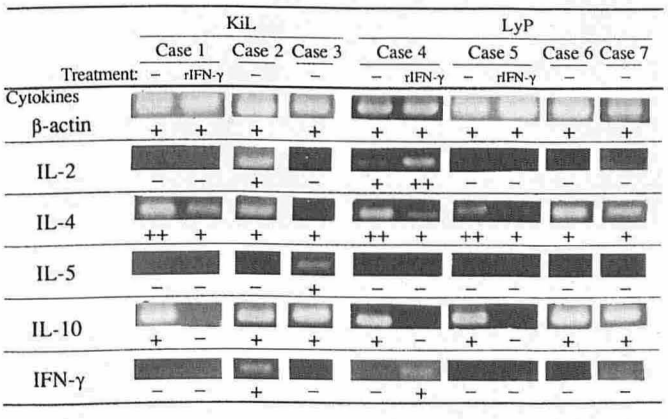

Figure 1. Th2 cytokine mRNA expression by dermal infiltrating cells of KiL and LyP is downregulated by predictive, local injection of rIFN-γ. Messenger RNAs were extracted from lesional dermis and analyzed by reverse transcriptase PCR. In cases 1, 2, 4, and 5, rIFN-γ at 2.5×10^4 JRU/cm^2 of skin surface, once a day, was injected into lesions for 3 consecutive days, and skin biopsy specimens were taken before (−) and after (rIFN-γ) injections. The intensity of mRNA expression was calculated in each sample according to the following formula: (density of cytokine band)/(density of β-actin band of the same sample). −, below the detectable level; +, detectable level; ++, density of the band was more than twice as strong as the rIFN-γ-treated or non-treated pair.

Table III. The Ameliorating Effect of Skin Test and Treatment With rIFN-γ Is More Apparent on CD30$^+$ Cells Than on CD3$^+$ Cells in the Lesional Skin[a]

	Skin Test: Number of CD3$^+$ Cells[b] (CD30/CD3)[c]		Treatment: Number of CD3$^+$ Cells (CD30/CD3)	
Cases	Before	After	Before	After
1	548 (0.94)	263 (0.55)	638 (0.96)	71 (0.16)
2	775 (0.99)	736 (0.96)	775 (0.99)	794 (0.97)
4	242 (0.91)	139 (0.47)	252 (0.93)	31 (0.04)
5	377 (0.82)	122 (0.32)	336 (0.88)	55 (0.14)

[a] Immunohistochemical study of skin biopsy specimens taken before and after the predictive skin test (intralesional injection, 2.5×10^4 JRU/cm^2 of skin surface, 3 consecutive days, once a day) and treatment (local or intravenous injection).

[b] The total number of positive cells in three 400× magnified fields in the center of cell infiltration was counted.

[c] Ratio of CD30$^+$ to CD3$^+$ cells in the same lesion.

neous CD30$^+$ lymphoproliferative disorders, but recent reports have demonstrated the association between CD30 expression and the production of Th2 cytokines in both CD4$^+$ and CD8$^+$ cells. Allergen-specific CD4$^+$CD30$^+$ T cells are stimulated to produce Th2 cytokines in the circulation of atopic patients after allergen exposure (Del Prete *et al*, 1995). Activated CD8$^+$ T-cell clones producing Th2 cytokines express membrane CD30 and release soluble CD30, whereas classic Th1-like CD8$^+$ T-cell clones do not (Manetti *et al*, 1994). Th2 cytokine mRNA expression of CD30$^+$ atypical cells in KiL and LyP seems to be in accordance with these findings. Consistent with a previous observation (Vowels *et al*, 1994), normal dermis contained mRNA for both IL-2 and IFN-γ, but not for IL-4 or IL-5 message. Because no known endogenous dermal cells produce IL-2 and IFN-γ, it is suggested that a small number of skin-infiltrating, activated T cells are primed to produce small amounts of these cytokines (Bos *et al*, 1987; Vowels *et al*, 1994). Although the exact source was unknown, IL-10 message detected in some of our samples might be derived from epithelial cells of hair follicles and sweat glands left in the dermis (Enk and Katz, 1992).

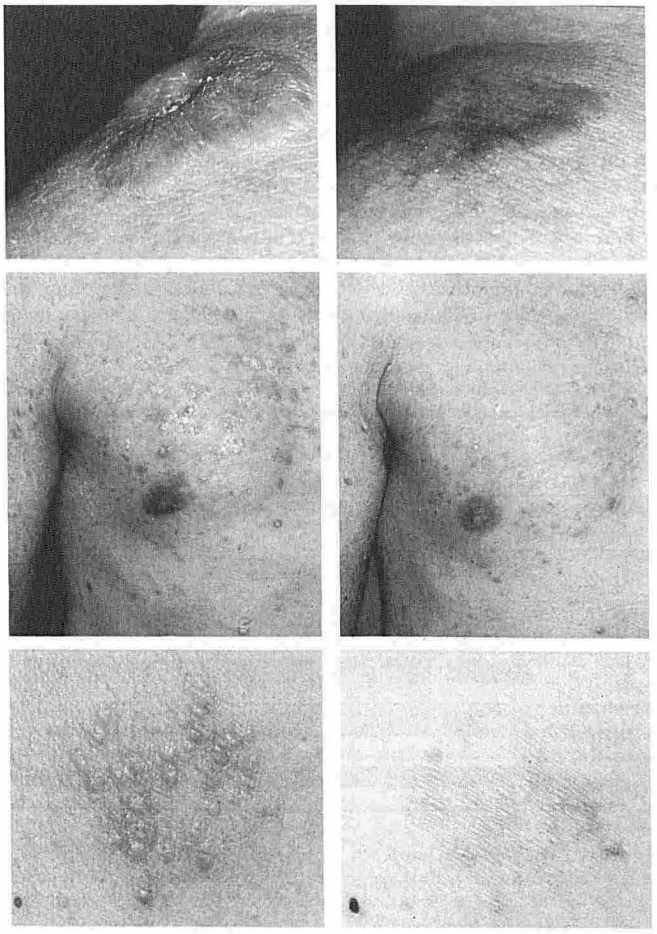

Figure 2. Skin lesions of KiL and LyP are improved by treatment with rIFN-γ. Clinical pictures of case 1 *(top)*, case 4 *(middle)*, and case 5 *(bottom)* before *(left)* and after *(right)* treatment with rIFN-γ show that the size of a tumor in KiL and the number and intensity of papules in LyP are decreased by rIFN-γ administration, done intravenously in cases 1 and 4 and locally in case 5.

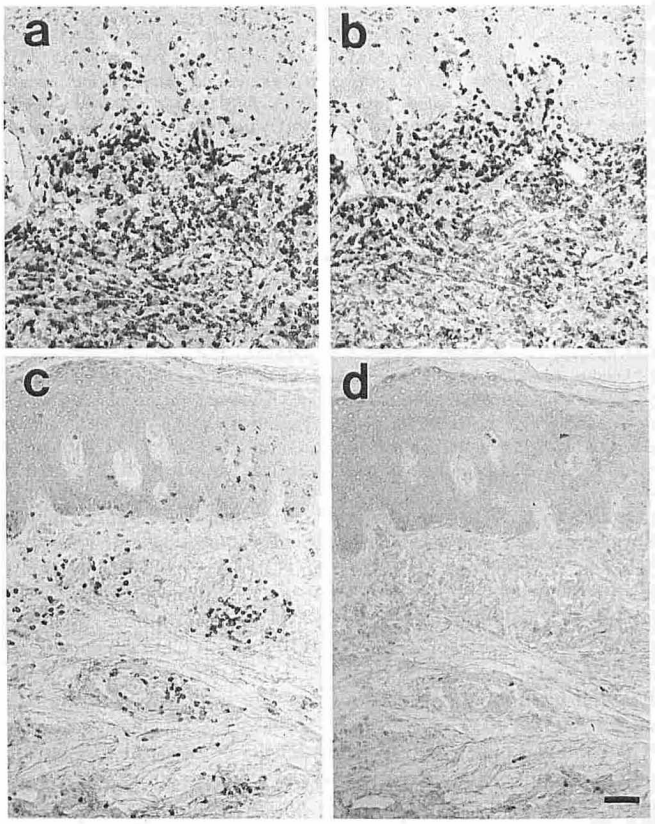

Figure 3. Treatment with rIFN-γ decreases the number of CD30$^+$ cells in the lesion. Immunohistochemical staining for CD3 *(a,c)* and CD30 *(b,d)* in the lesional skin of case 4 before *(a,b)* and after *(c,d)* rIFN-γ treatment shows a marked decrease in the number of infiltrating cells. The effect of rIFN-γ is more apparent on CD30$^+$ cells than on CD3$^+$ cells. *Scale bar*, 50 μm.

The presence of mRNA for IL-2 and IFN-γ in addition to Th2 cytokines in cases 2 and 4 raises several possibilities with regard to the cellular origin of these Th1 cytokines. Whereas skin-infiltrating cells exclusively expressed CD30 and the frequency of nontumorous inflammatory cells was low or absent compared with that of atypical cells, a subset of inflammatory cells might produce Th1 cytokines. Likewise, it is probable that mRNA for Th1 cytokines was expressed by dermal sessile components under pathologic conditions. In case 4, the finding that upregulation of mRNA expression for IL-2 and IFN-γ was associated with disappearance of CD30$^+$ cells favors the cited possibilities and argues against the possibility that atypical cells produce Th1 cytokines. In case 2, because of the lack of responsiveness to rIFN-γ, we also should assume that mRNAs for Th1 cytokines originated from CD30$^+$ cells; the tumor was harbored by one population that transcribed

both Th1 and Th2 cytokine mRNA or two populations that produced either Th1 or Th2 cytokines. The fact that lesional injection of rIFN-γ did not downregulate mRNA expression for Th2 cytokines seems to refute the latter possibility. If both types of cytokines were produced by one population, then CD30+ cells were the tumorous counterpart of Th0 cells. In any event, it is highly likely that CD30+ anaplastic cells are the source of Th2 cytokine messages.

The therapeutic efficacy of rIFN-γ for primary cutaneous CD30+ lymphoproliferative disorders was examined in two patients with KiL and two with LyP. Although the protocol was not double-blind or placebo-controlled, this preliminary study suggested that three of four cases responded well to rIFN-γ therapy. In cases 1 and 2, the skin lesions had been progressive from the beginning of the disease, with no tendency toward spontaneous regression. In LyP (cases 4 and 5), eruptions were waxing and waning before the treatment. To evaluate objectively the effect of rIFN-γ, we compared the tumor volume in KiL and scoring of eruptions in LyP before and after the treatment. Apparent objective improvement was noted in the responding patients. Furthermore, eruptions recurred within several weeks after discontinuation of rIFN-γ in these patients. Based on these results, we believe that the improvement was brought about by the treatment. Patients with KiL have been treated with local excision, radiotherapy, and systemic polychemotherapy, and patients with LyP show a partial response to psoralen plus UVA, topical mechlorethamine, and low doses of methotrexate (Willemze and Beljaards, 1993). Because most KiL has been reported to have a favorable prognosis and there is no satisfactory treatment for LyP, the administration of biologic response modifiers such as IFN-γ is the therapeutic choice for this recalcitrant group of cutaneous lymphoma, especially in aged patients. The mechanism by which IFN-γ inhibits the proliferation of CD30+ cells, although currently speculative, may involve the cooperative effects of two rIFN-γ actions. One action is direct, in which rIFN-γ downregulates the transcription of cytokine mRNA by CD30+ cells and, by doing so, as observed in cases 1, 4, and 5, inhibits the proliferation of these cells. In an indirect action, rIFN-γ enhances mRNA transcription in inflammatory Th1 cells, as illustrated by case 4, and these activated Th1 cells may exert anti-tumor cell activity.

CTCL is heterogeneous with respect to mRNA transcription and the production of cytokines. Both Th1- and Th2-type lymphoma cells are reported in Sezary syndrome and mycosis fungoides (Vowels *et al*, 1992, 1994; Borish *et al*, 1993; Saed *et al*, 1994; Tendler *et al*, 1994; Rook *et al*, 1995; Yagi *et al*, 1996). In Sezary syndrome, in which tumor cells belong to the Th2 type (Vowels *et al*, 1992, 1994; Borish *et al*, 1993; Saed *et al*, 1994; Tendler *et al*, 1994; Rook *et al*, 1995), peripheral blood mononuclear cells manifest a defect in production of IL-12 (Rook *et al*, 1995), which is a potent inducer of IFN-γ production (Chan *et al*, 1991) and Th1 cells (Sypek *et al*, 1993). The abnormal cytokine profile in these patients can be favorably altered by IL-12 (Rook *et al*, 1995). In mycosis fungoides, Th2 cytokine mRNA is detected in skin lesions at all stages of the disease, from the patch stage through the tumor stage (Vowels *et al*, 1994). These observations suggest a possible strategy for the treatment of Th2-type CTCL with Th1 cell–inducing modalities. In a recent phase II study in the United States, some patients with Sezary syndrome responded favorably to IFN-γ (Kaplan *et al*, 1990). Furthermore, IFN-α, which also enhances Th1 responses (Brinkmann *et al*, 1993), is extremely effective in the treatment of some CTCL (Olsen *et al*, 1989). On the other hand, we reported a case of Sezary syndrome with a Th1 cytokine profile (Yagi *et al*, 1996) in which peripheral blood mononuclear cells transcribed mRNA only for IL-2 and IFN-γ and produced these cytokines, but not IL-4, upon stimulation. Others have detected a Th1 cytokine profile at the plaque stage of mycosis fungoides (Saed *et al*, 1994). For Th1-type CTCL, treatment with IFN-γ may not be effective but rather may lead to deterioration because of stimulation of tumor cells. In this study, we examined the effects of lesionally injected rIFN-γ on atypical cells at the cytologic and genetic levels before the therapeutic trial. This skin test was safe and provided important information on predicting the effectiveness of rIFN-γ. Therefore, before rIFN-γ treatment, a predictive skin test in terms of the cytokine profile is needed in addition to categorization of cutaneous lymphoma.

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